

 **Stem cells**

- **Stem cells**
 - Body = about 200 different kinds of specialised cells, e.g. muscle, nerve, fat cells, skin cells
 - All cells in the body come from stem cells
 - A stem cell is a cell that is not yet (completely) specialised
 - Potential to differentiate → Process of specialisation = differentiation
 - Once the stem cell has become differentiated → the stem cell can no longer become another type of cell on its own
 - Stem cells used in adult for maintenance of organs and tissues
- **Pluripotent stem cells**
 - Can become any types of cells of the body (except placenta)
 - embryonic stem cells (ESC, ES cells); Terminology: **human** → **hESC**
 - induced pluripotent stem cells (iPSC, iPS cells); **human** → **hiPSC**
- **Multipotent**
 - Can become a few types of cells of the body
 - e.g.
 - mesenchymal stem cells (MSC)
 - hematopoietic stem cells (HSC)

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 **Stem cell therapy**

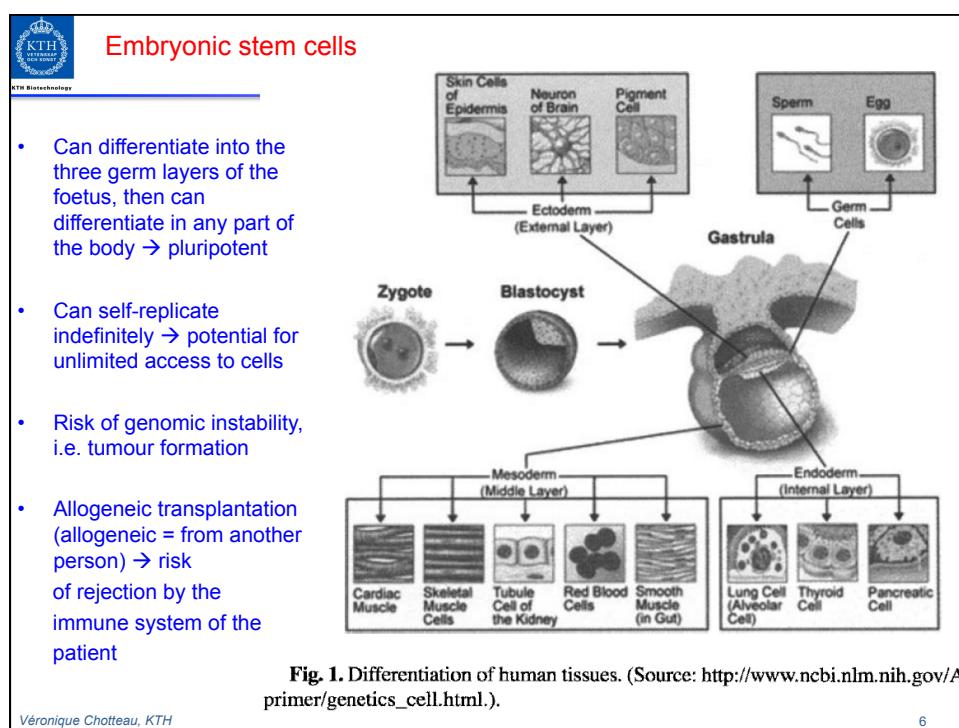
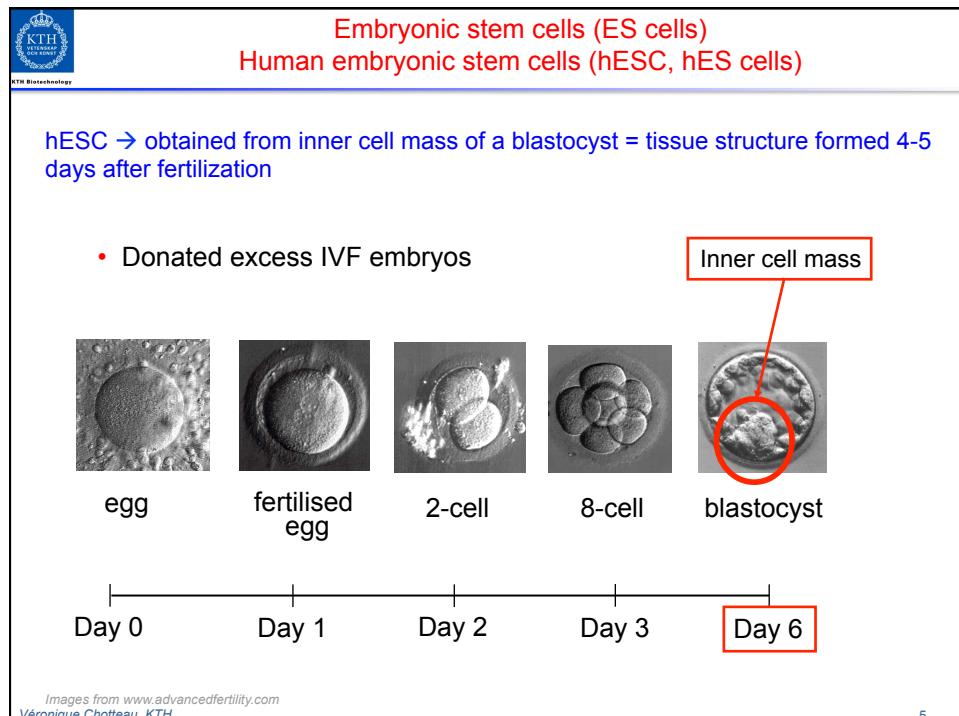
- **Challenges / issues**
 - Which system to use?
 - Safety
 - Keep functional cells in the body 
 - Autologous (own cells) vs. allogeneic (from donor)

autologous	allogeneic
no risk of immunogenic reaction	risk of immunogenic reaction
potentially more expensive	potentially less expensive
limitation in case of genetic disease (need reprogramming)	
	potentially access to large amount of cells
higher heterogeneity for production 	

 - Ethical concern 
 - Large scale manufacturing while keeping the correct cell fate

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Embryonic stem cells

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- Largest potential to replace body parts
- Difficult to maintain the cells in self-renewal state and avoid spontaneous differentiation
- Ethical issue
 - possibility to use parthenogenetic hESC = only mother genome
- Culture
 - traditionally need a feeder layer = helper for cell proliferation
 - new techniques to avoid feeder layer by extracellular matrix components
 - preference for culture without components of animal or human source to avoid virus/prion contamination and xeno-reaction (reaction against a foreign animal species)

Undifferentiated hESCs

Differentiated Cell Types

Differentiated Cell Types	Therapeutic Uses
Neural Cells	Spinal Cord Injury
Cardiomyocytes	Heart Failure
Islets	Diabetes
Dendritic Cells	Immunotherapy
Osteoblasts	Osteoporosis and Bone Fractures
Chondrocytes	Osteoarthritis
Hepatocytes	ADME Drug Testing

Geron has developed proprietary processes to convert hESCs into therapeutic cells.

Source: Tom Okarma – Geron, Stem Cell Policy Symposium, Stanford University 2009

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Induced pluripotent stem cells (iPSCs)

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- Induced pluripotent stem cells (iPSCs) → reprogrammed from differentiated fibroblasts in 2007 using Yamanaka factors
- Properties similar to hESC
 - Can differentiate into the three germ layers of the foetus, then can differentiate in any part of the body → pluripotent
 - Can self-replicate indefinitely → potential for unlimited access to cells
 - Risk of genomic instability, i.e. tumour formation
 - Largest potential to replace body parts
 - Difficult to maintain the cells in self-renewal state and avoid spontaneous differentiation
 - Culture → similar to hESC
- Transplantation
 - Allogeneic → risk of immunogenic reaction
 - Autologous → no risk of immunogenic reaction

Starting cells from donor tissue

Source: Australian Stem Cell Center

Induced change in gene expression

100μm

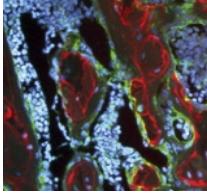
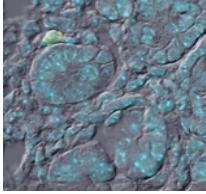
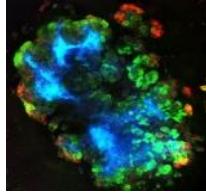
Human induced pluripotent stem cells cultured in electrospun fiber scaffold

Source KTH – C. Astrand, V. Chotteau

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Adult stem cells

- Adult stem cells = somatic cells = multipotent
 - present in lower number in tissues in specialized niches (special environment)
 - reside in most tissues of the body where they are involved in repair and replacement
 - e.g. bone marrow hematopoietic stem cells → can differentiate into the different blood cells

Bone marrow
Kidney
Lung

Source: Australian Stem Cell Center

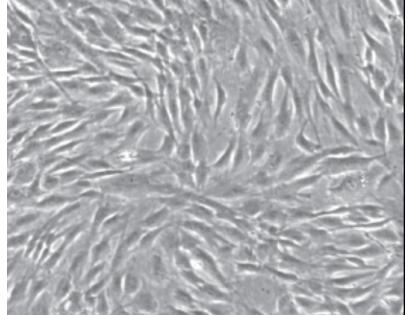
- Isolation from tissue → heterogeneous cell population (= several cell types at the same time)
 - possibility to sort the cells based on their cell surface markers (proteins) by e.g. fluorescence-activated cell sorting (FACS)
 - cells with limited potential for expansion → telomerase-dependent senescence
- Already used to treat patients (haematological malignancies, diseases of the immune system)

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Mesenchymal stem cells (MSCs)

- Stromal and non-hematopoietic cells
 - stroma = connective or structural role (i.e. no function) (while parenchyma = conduct the function of organs)
 - not from blood and not able to generate blood cells
- Source
 - bone marrow-derived MSCs (BM-MSCs)
 - adipose-derived MSCs (ASCs)
 - umbilical cord MSCs
 - tooth bud MSCs



Source: Irvine Scientific

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Mesenchymal stem cells (MSCs)

The diagram shows a vertical column of yellow circles representing stem cells in a bone marrow environment. One circle is labeled 'Osteoblast'. A red cell labeled 'MSC' is shown with a self-renewal cycle arrow. To the right, a central MSC cell is surrounded by arrows pointing to various differentiated cell types: Epithelial cell, Neuron, Connective stromal cell, Cartilage cell, Fat cell, Bone cell, Muscle cell, Gut epithelial cell, Lung cell, and another Osteoblast. These differentiated cells are grouped under three germ layers: Ectoderm (Epithelial cell, Neuron), Mesoderm (Connective stromal cell, Cartilage cell, Fat cell, Bone cell, Muscle cell), and Endoderm (Gut epithelial cell, Lung cell).

- Able to differentiate in cartilage, bone, adipose tissue, connective tissues, blood vessels, blood-related organs
- Multi-lineage potential: differentiation into various cell types, including adipocytes, hepatocytes, and neurocytes

Source: Uccelli et al 2008

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Mesenchymal stem cells (MSCs)

- Therapeutic aspects
 - Classically used for myeloma or lymphoma treatment (leucemia)
 1. Whole Body Irradiation → remove endogenous immune system and tumor
 2. Injection of bone marrow from donor → re-establish immune system
 - MSC useful as seed cells to replace damaged tissue in tissue engineering applications
 - Alleviate tissue injury and promote tissue repair by their anti-apoptotic and cytoprotective effects + angiogenic capacity
 - Promising approach to treat graft-versus-host disease (GVHD) and autoimmune disease due to immunomodulatory properties and low immunogenicity
 - MSCs release growth factors → therapeutically important paracrine function and effect on direct cell-cell interactions → may activate endogenous repair mechanisms
 - MSCs prevent anti-donor T-cell response → i.e. immunosuppressive milieu → generation of immune-privileged state

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Clinical

• Stem cells can be used differentiated or undifferentiated

- hESC and hiPSC should be differentiated to reduce the risk of tumor formation
- adult stem cells can be used differentiated or undifferentiated

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graph LR
    A[hESC Starting Material] --> B[Differentiation]
    B --> C[Frozen Final Product]
    C --> D[Central Manufacturing Facility]
    D --> E[Hospital]
    
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Source: Tom Okarma – Geron, Stem Cell Policy Symposium, Stanford University 2009

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Stem cell culture

State-of-the-art today in industry → 2D culture

- inefficient,
- high work load,
- expensive,
- limited scale-up,
- results in heterogeneous population
- risk for undesired differentiation

source: Pall

Brandenberger 2011

source: cellculturedish.com / Lonza

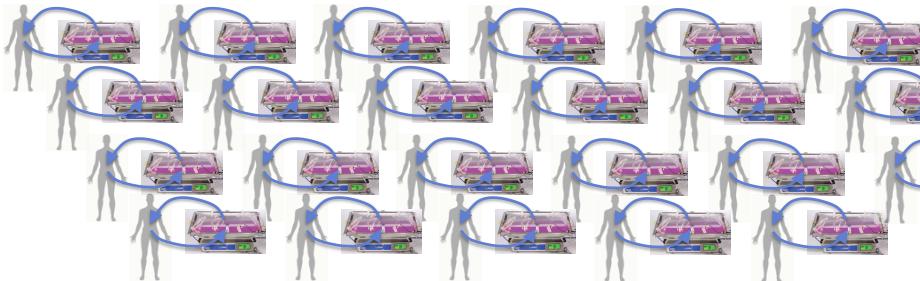
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Autologous therapies

- Autologous therapy = patient own cells are used to produce the therapy
- Large-scale production = scale-out 
- Personalised medicine

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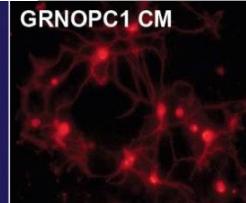
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Spinal Cord Injury

- GRNOPC1 (Geron)
- Derived from hESC → oligodendrocyte progenitor cells
- Improves locomotor activity
- Induces remyelination (rodents)
- Migrates through the spinal cord
- Produces neurotrophic factors
- Promotes neural outgrowth



Control



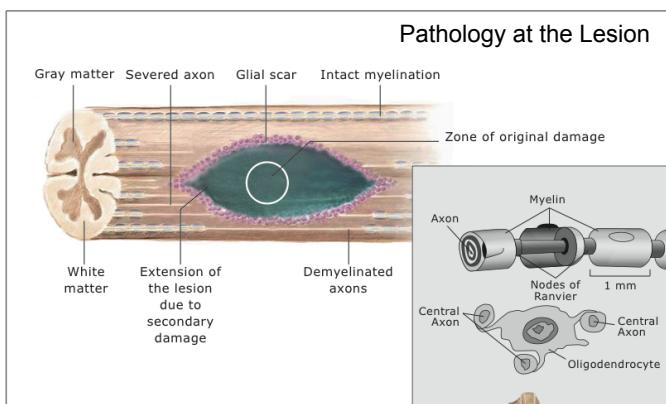
GRNOPC1 CM

2011: GERON aborted the program of GRNOPC1 ! 

2015 ≈ 20 on-going trial for demyelinating diseases and spine cord injuries

Source: Tom Okarma – Geron, Stem Cell Policy Symposium, Stanford University 2009

Pathology at the Lesion



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Treatment of deep cartilage defects in the knee

FDA approved cellular-based therapy for cartilage defects → chondrocytes

Autologous cells (biopsy) expanded ex vivo → large number of cells for transplantation

Problem = cells with tendency to dedifferentiate (loss of phenotype) in vitro

Influence of mechanical forces on cell function in vitro demonstrated for engineering cartilage and bones → need dynamic mechanical stresses on chondrocytes and MSC to promote differentiation and increase matrix production

The diagram illustrates the treatment of a knee cartilage defect. It shows a knee joint with a lesion. A biopsy of healthy cartilage is taken from the lesion. This biopsy undergoes enzymatic digestion and cultivation for 11-21 days, resulting in a 10-fold increase in cell number. The cells are then treated with trypsin and suspended in a tube containing $2.6 \times 10^6 - 5 \times 10^6$ cells. A periosteal flap is taken from the medial tibia and sutured over the lesion. Finally, cultured chondrocytes are injected under the flap into the lesion.

Source: M. Brittberg et al 1994
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Autologous stem cell therapy for thermal or chemical burns to the eye

- Corneal epithelial cells dissociated and expanded ex vivo.
- Cells taken from a patient's good eye (or from a small healthy section if the damage extends to both eyes)
- Limbal stem cells (LSCs) = responsible for the continuous regeneration and maintenance of the corneal epithelium.
- Over 2-4 weeks → patient's cells grown in vitro under GMP (good manufacturing practice)
- Finally grafted onto the limbus (narrow area located between the cornea and the conjunctiva) in the injured eye (or eyes) to replace the lost stem cells
- Product in India since 2008
- First stem cell therapy (Holoclar) since bone marrow transplantation approved (2015) for use in EU

Trial subjects → tracked up to 16 years

- Future: iPSCs = promising option → corneal epithelial cells

Casaroli-Marano et al J Clin Med. 2015 Feb; 4(2): 318-342
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The diagram shows a cross-section of the eye with labels: cornea, limbus, and conjunctiva. A white rectangular box indicates the area where stem cells are grafted onto the limbus. An inset labeled 'a' shows a close-up of the eye with the same anatomical regions labeled.

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Cardiac function restoration

- Cell based therapies generating new cardiomyocytes and vessels
- Cells isolated from bone marrow (MSC), cardiosphere-derived cell (cultured from heart biopsy) or cardiac stem cells (heart)
→ autologous or allogenic
- Status: under clinical trials, not commercial
- Safety = OK but outcome uncertain sometimes improvement, sometimes not
→ suggests potential of cell-based therapies to reduce cardiac scar size and to improve cardiac function in patients with ischemic cardiomyopathy.

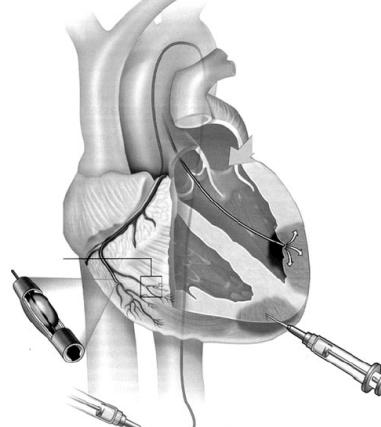


FIG. 1. Delivery options for cardiac cell therapy. MSCs can be directly injected into the diseased heart via the epicardium (surgical approach) or transendocardially (usually requiring imaging guiding). Intracoronary injection can also be performed. Not shown here is systemic, intravenous MSC application, which requires active migration of cells to the heart.

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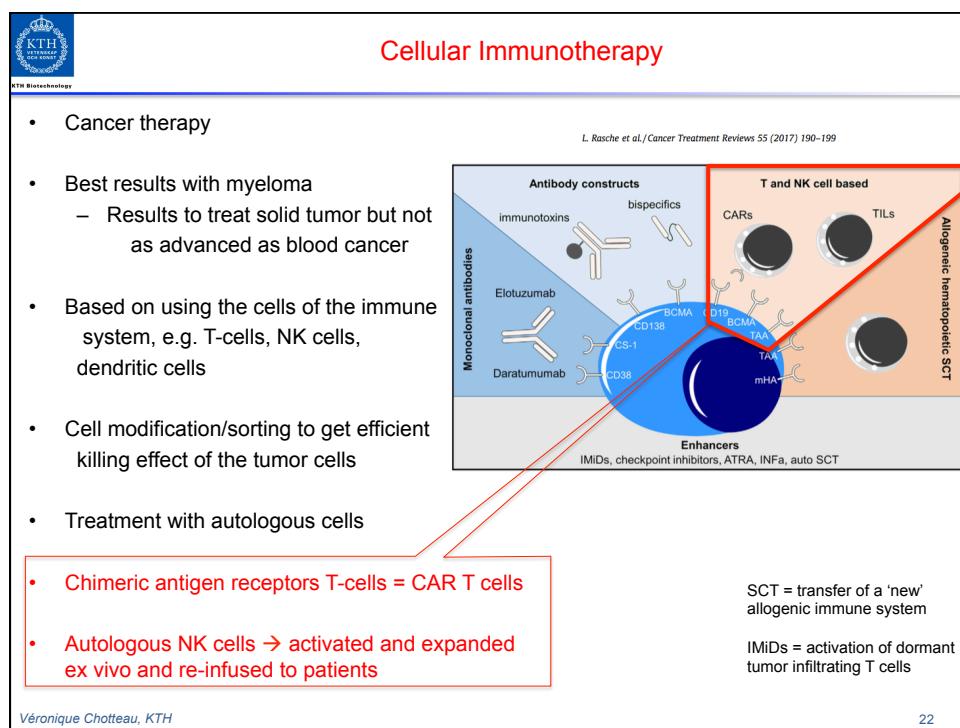
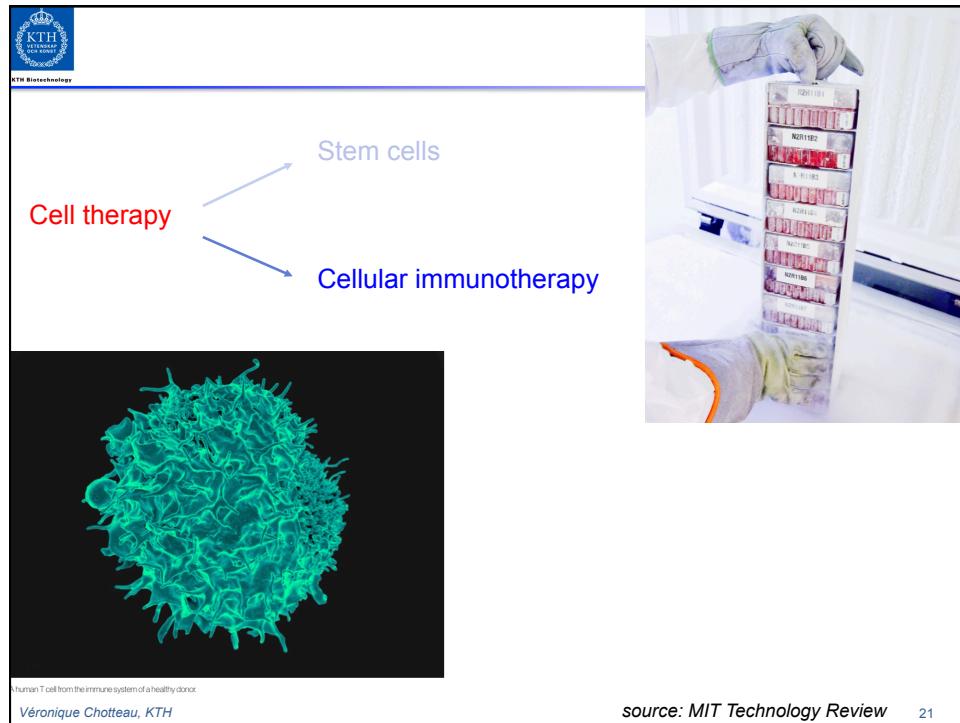
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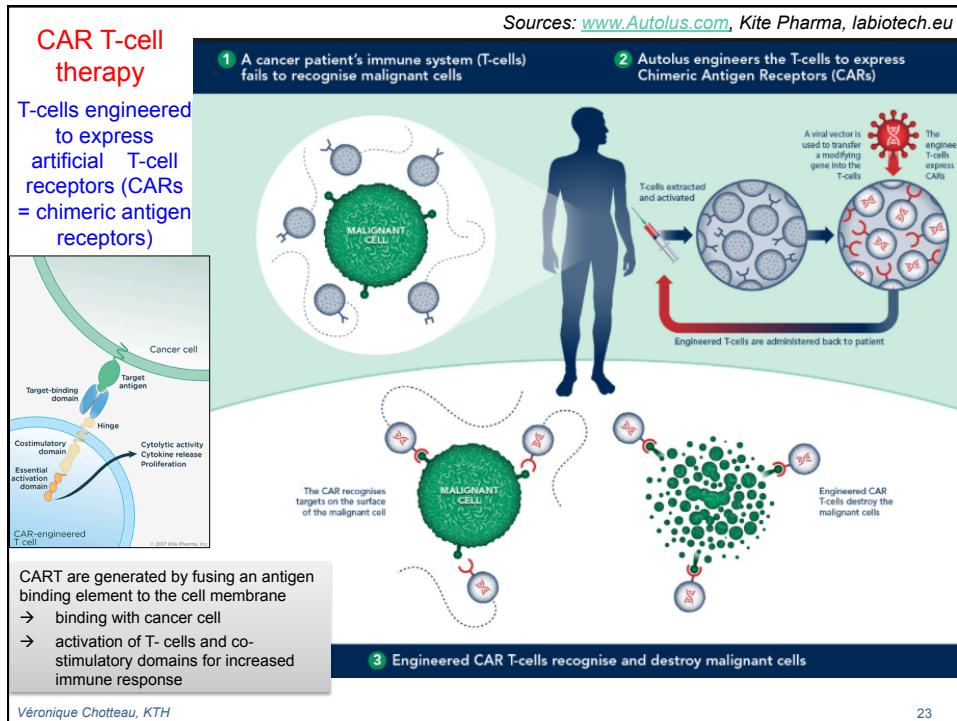
FDA approved cellular and gene therapy products

ALLOCORD (HPC Cord Blood)	hematopoietic and immunologic reconstitution
LAVIV (Azfice-T, autologous fibroblasts)	moderate to severe nasolabial fold wrinkles in adults
MACI (Autologous Cultured Chondrocytes on a Porcine Collagen Membrane)	repair of cartilage defects of the knee
CLEVECORD (HPC Cord Blood)	hematopoietic and immunologic reconstitution
GINTUIT (Allogeneic Cultured Keratinocytes and Fibroblasts in Bovine Collagen)	allogeneic cellularized scaffold - vascular wound mucogingival
HEMACORD (HPC, cord blood)	hematopoietic and immunologic reconstitution
Ducord, HPC Cord Blood	hematopoietic and immunologic reconstitution
HPC, Cord Blood	hematopoietic and immunologic reconstitution
HPC, Cord Blood - MD Anderson Cord Blood Bank	hematopoietic and immunologic reconstitution
HPC, Cord Blood - LifeSouth	hematopoietic and immunologic reconstitution
HPC, Cord Blood - Bloodworks	hematopoietic and immunologic reconstitution
IMLYGIC (talimogene laherparevec, gene therapy)	local treatment in patients with melanoma recurrent after initial surgery
KYMRIAH (tisagenlecleucel)	CAR-T fro acute lymphoblastic leukemia
LUXURNA (gene therapy)	adeno-associated virus vector-based gene therapy - patients with retinal dystrophy
PROVENGE (sipuleucel-T)	metastatic prostate cancer
YESCARTA (axicabtagene ciloleucel)	CAR-T for follicular lymphoma

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CAR T-cell therapy

- Chimeric antigen receptors-modified T cells = T cells engineered to express receptors specific to the particular form of cancer → can target any tumor → T cells can then recognize and kill the cancer cells
 1. Collection of a patient's T cells
 2. Cells genetically engineered to express receptor directed towards antigens on the tumor cells
 3. The cancer-killers cells → put back inside the same patient and once they find their target they multiply
- Promising anti-cancer therapy
 - ≥ 300 clinical trials
- Potentially need of cell suicide or elimination switches due to over expansion of transplanted T cells
 - e.g. trigger of cell suicide (apoptosis) by small molecule

First cancer 'living drug' gets go-ahead

By James Gallagher
Health and science reporter, BBC News website

30 August 2017 | Health



The US has approved the first treatment to redesign a patient's own immune system so it attacks cancer.

The regulator - the US Food and Drug Administration - said its decision was a "historic" moment and medicine was now "entering a new frontier".

The company Novartis is charging \$475,000 (£367,000) for the "living drug" therapy, which leaves 83% of people free of acute lymphoblastic leukaemia.

Most patients respond to normal therapy and Kymriah has been approved for those where treatments fail.

Dr Stephan Grupp, who treated the first child with CAR-T at the Children's Hospital of Philadelphia, said the new approach was "enormously exciting".

"We've never seen anything like this before," he added.

That first patient had been near to death, but has now been cancer-free for more than five years.

Out of 63 patients treated with CAR-T therapy, 83% were in complete remission within three months and long-term data is still being collected.

